



## Current Opinion

## Discovery of veterinary antiparasitic agents in the 21st Century: A view from industry

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## ARTICLE INFO

## Article history:

Received 1 March 2010

Received in revised form 15 April 2010

Accepted 19 April 2010

## Keywords:

Drug discovery

Animal health

## ABSTRACT

Discovery of antiparasitic agents is a challenging process, requiring discovery of molecules with the ability to kill parasites but not their hosts. Customer preference is for fewer doses and ease of application, but this is not always compatible with reduced withdrawal times, human food safety and/or user safety. This article describes some of the difficulties faced by researchers in the search for new antiparasitic agents, while highlighting advances that may improve the discovery process and the chance of success in discovering novel drugs.

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## 1. Why do we need new antiparasitic drugs in the Animal Health industry?

The two major markets for animal health drugs, companion animals and livestock, each have unmet needs for antiparasitic drug discovery. The companion animal antiparasitic market (dog/cat) has grown to a value of \$3.4 billion in 2008 (Vetnosis). The high value of the market has been driven by development of highly efficacious agents for treating ectoparasites, with the introduction of fipronil (1994) and imidacloprid (1996). Currently, treatments are divided principally into ecto- or endo-parasitic profiles, with the exception of endectocidal products such as Revolution™ (Selamectin) and Advocate™ (Imidacloprid and Moxidectin).

Emerging evidence of resistance in fleas (Daborn et al., 2004), and reports of refractoriness of heartworm agents (American Heartworm Society Canine Guidelines <http://www.heartwormsociety.org/veterinary-resources/canine-guidelines.html#4>) may impact current products on the market. Researchers recently highlighted incidences of heartworm preventative failures that could not be explained by non-compliance and studies are being initiated to investigate this further (Blagburn et al., 2010). Characterisation of heartworm prevention failures in the central United States. 13th Triennial State of the Heartworm Symposium (American Heartworm Society), Memphis, Tennessee, USA. Although there is no evidence to date that the reports of lack of efficacy are due to resistance. Moreover, there are increasing worries among pet owners about disease transmission by ticks. Delivery of drug is another major concern for pet owners and veterinarians; so alternative

delivery routes and technologies may present opportunities such as ease of administration, accuracy of dosing and avoidance of environmental contamination.

The livestock antiparasitic market had a value of \$1.8 billion in 2008 (Evans et al., 2009). Macrocyclic lactones (MLs) are widely used to control ecto- and endoparasites in livestock. There are, however, concerns over emergence of resistance; resistance to MLs in sheep nematodes has already dramatically reduced their utility in many regions (Sangster, 1999), and there are growing reports of failures of MLs to control cattle nematode parasites, particularly in Latin America and New Zealand (Mejia et al., 2003; Jackson et al., 2006; Wrigley et al., 2006). There have also been suggestions of ML cross-resistance against synthetic pyrethroid-resistant flies (Scott, 1989). There is still, therefore, considerable scope for new antiparasitic drugs to be delivered to both the companion animal and livestock markets.

Of concern, however, is the increasingly rapid contraction of the industry in recent years (Fig. 1). Of 35 Animal Health companies in 1990, there are now less than 10. It is difficult to put a firm figure on the impact on investment in antiparasitic discovery, but historically the experience has been that mergers usually focus on cost savings and rarely lead to increased investment. There is little doubt that this level of consolidation will lead to less competition, which may translate into fewer chemical classes being discovered.

## 2. How do we design a quality veterinary antiparasitic drug?

While there is no such thing as the 'ideal' drug, there are some criteria that are important for development of a successful antiparasitic agent (Woods and Williams, 2007).

Control of commercially important parasites is essential and, in animal health, spectrum is critical. Both farmers and pet owners

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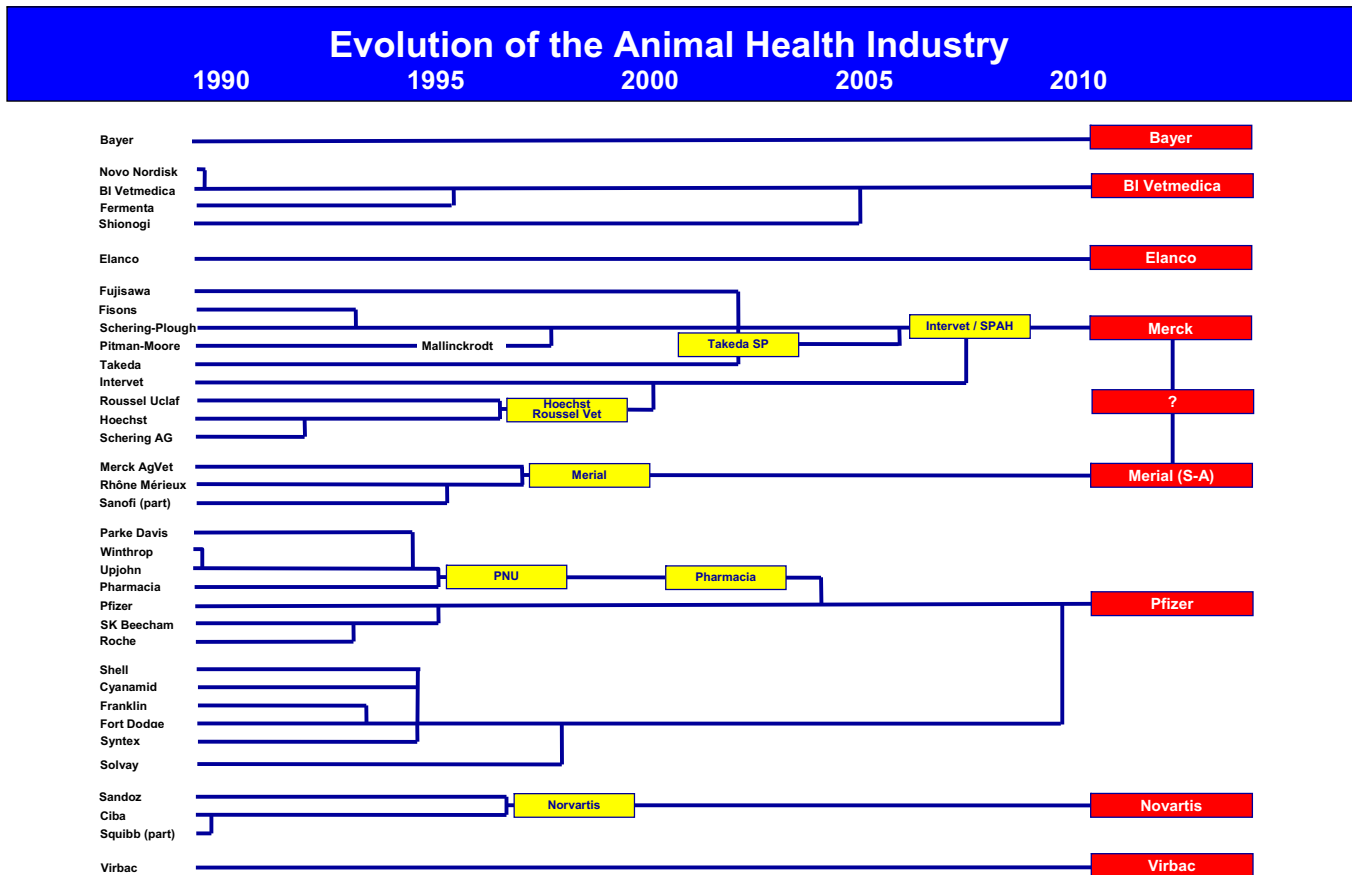


Fig. 1. A diagrammatic representation of the consolidation which has occurred over the last 20 years in the Animal Health industry. Courtesy of Richard O. Nicholas (Pfizer Ltd., Sandwich, United Kingdom).

want to dose their animals as few times as possible, so the more parasites controlled by a single drug the better. The MLs set a high standard, but even endectocides have spectrum gaps. At the least, a new drug cannot offer less control than existing products, unless it presents another overriding benefit. Similar issues are considered for companion animal products, although that market is driven by quality of life and owner perception of the impact of parasites on their pets and themselves. Resistance is always a major driver of new antiparasitic drug development and, although more of an issue for some parasites and drugs than for others, emergence of resistance is a constant challenge. Particularly problematic is the requirement for persistence to minimise dosing frequency; an important advantage to both pet owners and farmers. This has to be balanced by the often opposing need to consider the safety to the animal being dosed as well as human food safety for livestock.

Regulatory requirements are also essential for defining the properties of any new drug. For example, according to the European Agency for the Evaluation of Medicinal Products guidelines (<http://www.ema.europa.eu/pdfs/vet/ewp/000500-rev.2.pdf>), acaricidal agents must cause detachment and death of ticks within 24–48 h of infestation, without having had a blood meal. Study design has to take this into consideration.

Although efficacy is central to the success of a drug, there are many other parameters that impact on the successful development of a drug. Chiral centres are common in pharmaceutically active compounds, resulting in racemic mixtures of enantiomers or even diastereoisomers. If activity is found only in one enantiomer, developing a stereoselective synthetic route, or chiral separation, may be required. Formulation is key to delivering a drug effectively to the site of action. Complicating this in animal health is the fact that, depending on the end user, the delivery route can be oral, top-

ical or by injection; each route offering different challenges with respect to formulation. Despite these hurdles, formulation scientists are stepping up to the challenge and making a significant impact on our ability to deliver antiparasitic agents with the desired pharmacokinetic profile while still considering ease of application, safety of the formulation, dose volume and stability. Pharmacokinetic/pharmacodynamic (PK/PD) analyses and modelling are proving to be very powerful tools in the development of pharmaceuticals (Toutain, 2002). PK/PD analysis helps determine the relationship between the absorption, distribution, metabolism and elimination (ADME) properties of a drug and the physiological response to the drug and modelling generates better predictions of drug action and optimal dose regimen.

Once a formulation has been developed, there is still a major hurdle to overcome regarding stability and shelf life. Drug stability can be assessed early using accelerated drug stability protocols and ultraviolet light stability is also easily measured. Packaging can help to manage some issues of formulated drugs, for example by excluding water or light. Polymorphism is the ability of a compound to exist in more than one form or crystal structure and it can derail a drug development programme if the most stable polymorph is not identified early. As different polymorphs often have different solubilities and compression qualities, efficacy of the final formulation can be affected, so clinical studies need to identify and utilise the final drug form.

Safety is a vital component of delivering a new antiparasitic drug. Antiparasitic drug discovery scientists have a unique challenge in delivering safe drugs, as their aim is to discover toxic compounds. Ideally, starting with a drug target that is specific to the parasite helps in identifying safer molecules; the new anthelmintic drug class, the aminoacetonitrile derivatives (AADs), are an exam-

ple where the target is a nematode-specific nicotinic acetylcholine receptor subunit, *acr-23* (Kaminsky et al., 2008). Having a parasite-specific target is not, however, essential and there are examples where a safe therapeutic index can be generated by selectivity at the receptor. For example, the neonicotinoids are selective for insect nicotinic acetylcholine receptors (Matsuda et al., 2001; Tomizawa and Casida, 2005) and phenylpyrazoles have selectivity for insect GABA-gated Cl<sup>-</sup> channels (Hainzl et al., 1998), although the latter is complicated by activity at insect glutamate-gated Cl<sup>-</sup> channels (Narahashi et al., 2010). In addition to an acceptable therapeutic index, safety evaluation of veterinary drugs requires investigation of adverse reactions, species- and breed-specific effects, mutagenicity/carcinogenicity, drug:drug interactions, human food safety (for food animals) and handler safety. Human food safety can have a significant impact on the withdrawal time of a drug, which might make it unacceptable in the market. Environmental safety is also a regulatory requirement.

It is a disagreeable reality that profitability drives discovery of all commercially successful agents; all businesses must generate a return on investment to survive long-term and the pharmaceutical industry is no different. What this means is that the bigger the predicted value of a market, the higher the investment in drug discovery and the greater the risk that can be entered into. Predictions of the value of a product that may not be launched for 10 years are fundamental in determining the level of investment in lead-seeking, although the difficulties of generating accurate forecasts are recognised. Once it is agreed that a product profile has value, there are still cost concerns to consider throughout the lifetime of the drug discovery and development process. Cost of drug synthesis is critical for determining efficacious dose and the synthetic route must be scalable and not include any dangerous processes. Considerations of whether a manufacturing facility capable of synthesising the drug is available or would have to be constructed also have to be addressed early in the discovery process.

### 3. How do we find leads?

As discussed in previous articles (Woods and Williams, 2007; Geary et al., 2009), all antiparasitic drugs have been discovered by empirical screening in parasites or animal models (Campbell, 1993), with mechanisms only identified retrospectively (Kaminsky et al., 2008). This is still a valid approach to the discovery of novel antiparasitic molecules, though not necessarily compatible with the large size of pharmaceutical company files. Despite not being comparable with true high-throughput screening, significant screening can be conducted against the parasite *in vitro* using directed approaches and, in reality, all antiparasitic molecules must be tested against the parasite before progression to animal models (Conder et al., 1990), so predictive *in vitro* models are important. Technologies, both biochemical and imaging (Feng et al., 2004; Hördegen et al., 2006), are emerging and are increasingly being applied to screening methodologies, but still need to demonstrate similar sensitivity and predictive capability to assays measuring phenotype or motility as an endpoint (Kotze et al., 2004; Schürmann et al., 2007; Moy et al., 2009).

Despite being an unproven approach, there are definite advantages in mechanism-based approaches to the discovery of antiparasitic drugs. Identifying molecules with activity against a mechanistic target eliminates issues with delivery of compound to the parasite – penetration, metabolism and elimination. Generating quantitative concentration-activity data aids in better understanding of structure-activity relationships and optimisation of activity at the receptor level. Chemists can then focus on resolving issues with penetration, metabolism, etc. as separate issues, while ensuring any changes do not impact on activity at the receptor.

Additionally, Animal Health companies often have access to large files of compounds, either through parent human pharmaceutical companies or by leveraging small molecule or natural product files owned by small biotechnology companies. It would be a missed opportunity not to attempt to leverage these molecules, with either whole parasite or mechanism-based screens.

The next question is whether to be cautious and explore established chemical space and/or validated targets, or to seek novelty. A balanced portfolio, where precedented and ‘first principle’ approaches are investigated in parallel, is generally considered a successful strategy. This allows for risk to be managed by focusing on areas with a higher chance of success; while still endeavouring to discover the next new target or molecule.

Despite the lack of success of targeted approaches historically, we do believe in a balanced approach to discovery of new substrate for antiparasitic agents. Key to success in this endeavour will be to recognise the mistakes of the past and ensure future efforts focus on identifying the best leads for progression. Quality of compound libraries is central to success or failure of targeted approaches and companies such as Pfizer are investing in file enrichment programmes to improve library quality and thereby efficiency of high-throughput screening campaigns. As we discuss later, the initial assay design is also key to success and we highlight some important features of predictive mechanism-based assays. For success in antiparasitic discovery, it is also important to build an understanding of physicochemical and pharmacokinetic properties which are important for translation from mechanism-based assays to antiparasitic activity.

### 4. Target validation

A ‘validated’ target is one for which a drug is on the market and is successfully treating the disease; but it might also be regarded as one for which a molecule exists which has been shown to deliver efficacy by acting at this target. Target identification is often not straightforward, however, and there can be debate over many years before a drug target is finally confirmed (pharmacology is rarely ‘monospecific’ and, depending on the dose, a drug may have activity at number of receptors). For a long time, the macrocyclic lactones were thought to act through agonism of GABA-gated Cl<sup>-</sup> channels, based on activity at mammalian receptors (Pong and Wang, 1980), until it was finally shown that antiparasitic activity is mediated through the glutamate-gated Cl<sup>-</sup> channel (Arena et al., 1995; Cully et al., 1996; Delany et al., 1998; Wolstenholme and Rogers, 2005). Novel targets were historically identified after a new molecule with antiparasitic activity had been discovered. This is still a valid approach and genomics approaches allow this to be rapidly achieved (Kaminsky et al., 2008). Genomics technologies with model organisms (*Caenorhabditis elegans* and *Drosophila melanogaster*) have also opened up new avenues for target identification and validation in the absence of a molecule with activity at the receptor (McCarter, 2004; Gilleard et al., 2005; Woods and Williams, 2007), although similar approaches with parasitic nematodes are proving a challenge (Knox et al., 2007).

### 5. Assay design and hit discovery

Once an approach has been decided, an assay must be developed and characterised. Given that marketed antiparasitic drugs consist of compounds that exhibit both agonist (e.g. MLs) and antagonist (e.g. fipronil) pharmacology, validating assays that measure compound function is of critical importance. An essential consideration for agonist-based drug discovery is the ability of the functional assay to accurately measure both potency and intrinsic efficacy (i.e. agonist versus partial agonist) of the compound of

interest. The pharmacological effect of any agonist is a function of its intrinsic efficacy and the extent to which it occupies the target. Therefore, accurately measuring whether a compound behaves as a full or partial agonist will allow for a better prediction of the compound's ability to demonstrate antiparasitic activity through activation of a given receptor system. It is important to consider that as medicinal chemistry explores the structure–activity relationship (SAR) it is likely that compound analoguing will generate molecules exhibiting a dynamic range of intrinsic efficacies. Complicating this analysis is the ability to work in recombinant systems that mitigate the classical effects of “spare receptors” (i.e. receptor reserve) on agonist behaviour. Under conditions of receptor reserve, potencies of full/partial agonists and intrinsic efficacies of partial agonists increase as reserve increases, potentially leading to overestimates of agonist activity. Identifying the receptor density levels in the many parasites of interest is clearly a difficult task, thus complicating the understanding of what the appropriate receptor density level should be in a recombinant expression system. Therefore, it is best to validate assays using recombinant expression systems that produce a dynamic range of intrinsic efficacies and correlate those with activity in whole organism assays. An additional factor to consider beyond the relative efficacy of a compound is its capacity to alter the activation kinetics of a given receptor. For instance, the antiparasitic activity of pyrethroids has been linked to their ability to induce marked changes in Na<sup>+</sup> channel activation and inactivation kinetics. Specifically, electrophysiology studies have confirmed that pyrethroids slow the kinetics of opening and closing of Na channels at the molecular level, thus impacting the insect nervous system by slowing action potential decay (Vais et al., 2001). When targeting ion channel agonists, the validated assay must accurately measure not only the relative efficacy of the molecules, but also their ability to modulate receptor kinetics. For antagonist-driven drug discovery, validated assays are more focused on measuring potencies to block the receptor and the ability to understand the nature of that blockade (e.g. competitive versus non-competitive inhibition). However, when exploring compounds that inhibit voltage-gated ion channels, the capability of the assay to measure state-dependent block becomes important. For instance, the sodium channel blocker insecticides (e.g. pyrazolines) have been shown to have no effect at hyperpolarized membrane potentials but cause blockade of the channel as the membrane potential is depolarised (Silver et al., 2010). Therefore, the ability of an assay to accurately control membrane potential changes is of critical importance in assessing voltage-dependent antagonists. While the use of cell-based assays with membrane potential dyes or ion-flux measurements is widely available, they represent indirect measures of ion-channel activity and often generate data that does not correlate well with electrophysiological techniques. With the advancement of various automated electrophysiology platforms e.g. IonWorks™, Molecular Devices, (<http://www.moleculardevices.com/pages/instruments/ionworks.html>), strong consideration should be given to utilising these systems when evaluating voltage-dependent mechanisms.

Once an assay has been developed, miniaturisation (in 384-, 1536 or even 3456-well assay plates) allows millions of compounds to be tested against the target. This rapidly identifies active molecules for progression to in vitro parasite assays and to lead optimisation programs. A major bottleneck for veterinary antiparasitic screening in the past, high-throughput screening using parasite or model-organism targets is now standard. Recombinant targets are increasingly more accessible, with the number of published genomes growing and genetic technologies improving, allowing functional screens using parasite targets to become achievable; with the caveat that recombinant expression of some invertebrate ion channels has proved challenging.

## 6. Lead optimisation

The next hurdle is to translate activity at the receptor into in vitro and then in vivo antiparasitic activity in the host animal. Scientists often find themselves addressing a number of issues in parallel such as potency, penetration, chemical and metabolic stability, solubility, spectrum, PK/PD and therefore must have a clear strategy for identifying and addressing the issues. A great deal of substrate is lost at this stage, highlighting the reality that efficacy at the receptor, or even antiparasitic activity against the parasite in vitro, does not in itself create a drug. High-content parallel chemistry technologies (Edwards, 2006, 2009) allow for rapid analoguing and exploration of chemical space and SAR. Computational chemistry is also becoming increasingly more predictive and can aid in drug design, as well as allowing for virtual screening, where applicable (Walters et al., 1998).

If the antiparasitic drug discovery team overcomes all the odds and discovers a new chemical entity which matches with the product profile for a successful veterinary antiparasitic agent, the team will then embark on ensuring the drug meets the requirements set out in Section 2. If possible, some of the key issues will have been addressed such as formulation, cost of goods, chirality and polymorphs during the lead optimisation process.

Antiparasitic drug discovery faces considerable hurdles, with increasing regulatory requirements and increasing demands from the marketplace for the next blockbuster; but the Animal Health industry approaches the challenge with new technologies and strategies that will help in identifying and developing higher quality molecules.

## Acknowledgement

The authors would like to thank Richard O. Nicholas for his help with accessing antiparasitic market information and for constructing Fig. 1.

## References

- Arena, J.P., Liu, K.K., Pares, P.S., Frazier, E.G., Cully, D.F., 1995. The mechanism of action of avermectins in *Caenorhabditis elegans*: correlation between activation of glutamate sensitive chloride current, membrane binding and biological activity. *Journal of Parasitology* 81, 286–294.
- Blagburn, B., Dillon, R., Prichard, R., Geary, T., Mount, J., Land, T., Butler, J., Bourguinat, C., 2010. Characterisation of heartworm prevention failures in the central United States. In: 13th Triennial State of the Heartworm Symposium (American Heartworm Society), Memphis, Tennessee, USA.
- Campbell, W.C., 1993. Ivermectin, an antiparasitic agent. *Medicinal Research Reviews* 13, 61–79.
- Conder, G.A., Jen, L.-W., Marbury, K.S., Johnson, S.S., Guimond, P.M., Thomas, E.M., Lee, B.L., 1990. A novel anthelmintic model utilizing jirds, *Meriones unguiculatus*, infected with *Haemonchus contortus*. *Journal of Parasitology* 76 (2), 168–170.
- Cully, D.F., Pares, P.S., Liu, K.K., Schaeffer, J.M., Arena, J.P., 1996. Identification of a *Drosophila melanogaster* glutamate-gated chloride channel sensitive to the antiparasitic agent avermectin. *Journal of Biological Chemistry* 271, 20187–20191.
- Daborn, P., McCart, C., Woods, D., French-Constant, R., 2004. Detection of insecticide resistance-associated mutations in cat flea *Rdl* by TaqMan-allele specific amplification. *Pesticide Biochemistry & Physiology* 79 (1), 25–30.
- Delany, N.S., Laughton, D.L., Wolstenholme, A.J., 1998. Cloning and localisation of an avermectin receptor-related subunit from *Haemonchus contortus*. *Molecular and Biochemical Parasitology* 97, 177–187.
- Edwards, P.J., 2006. The impact of parallel chemistry in drug discovery. *Drugs* 9 (5), 348–353.
- Edwards, P.J., 2009. Current parallel chemistry principles and practice: application to the discovery of biologically active molecule. *Current Opinion in Drug Discovery & Development* 12 (6), 899–914.
- Evans, T., Chapple, N., van den Driesche, J., Lloyd, J., 2009. Parasiticides. *Vetnosis*, Edinburgh, UK.
- Feng, Z., Cronin, C.J., Wittig, J.H., Sternberg, P.W., Shafer, W.R., 2004. An imaging system for standardized, quantitative analysis of *C. elegans* behaviour. *BMC Bioinformatics* 5, 115–120.
- Geary, T., Woods, D.J., Williams, T.M., Nwaka, S., 2009. The Target identification and mechanism-based screening for anthelmintics: application of veterinary antiparasitic research programmes to search for new antiparasitic drugs for

- human indications. In: Selzer, Paul (Ed.), *Drug Discovery in Infectious Diseases*. Wiley-VCH, Verlag GmbH, Weinheim, pp. 3–15.
- Gilleard, J.S., Woods, D.J., Dow, J.A.T., 2005. Model organism genomics in veterinary parasite drug discovery. *Trends in Parasitology* 21 (7), 302–305.
- Hainzl, D., Cole, L.M., Casida, J.E., 1998. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfonyl photoproduct. *Chemical Research in Toxicology* 11 (12), 1529–1535.
- Hördegen, P., Cabaret, J., Hertzberg, H., Langhans, W., Maurer, V., 2006. *In vitro* screening of six anthelmintic plant products against larval *Haemonchus contortus* with a modified methyl-thiazolyl-tetrazolium reduction assay. *Journal of Ethnopharmacology* 108 (1), 85–89.
- Jackson, R., Rhodes, A.P., Pomroy, W.E., Leathwick, D.M., West, D.M., Waghorn, T.S., Moffatt, J.R., 2006. Anthelmintic resistance and management of nematode parasites on beef cattle-rearing farms in the North Island of New Zealand. *New Zealand Veterinary Journal* 54 (6), 289–296.
- Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Schorderet Weber, S., Wenger, A., Wieland-Berghausen, S., Goebel, T., Gauvry, N., Pautrat, P., Skripsky, T., Froelich, O., Komoin-Oka, C., Westlund, B., Sluder, A., Mäser, P., 2008. A new class of anthelmintics effective against drug-resistant nematodes. *Nature* 452, 176–180.
- Knox, D.P., Geldhof, P., Visser, A., Britton, C., 2007. RNA interference in parasitic nematodes of animals: a reality check? *Trends in Parasitology* 23 (3), 105–107.
- Kotze, A.C., Clifford, S., O'Grady, J., Behnke, J.M., McCarthy, J.S., 2004. An *in vitro* larval motility assay to determine anthelmintic sensitivity for human hookworm and *Strongyloides* species. *American Journal of Tropical Medicine and Hygiene* 71 (5), 608–616.
- Matsuda, K., Buckingham, S.D., Kleier, D., Rauh, J.J., Grause, M., Sattelle, D.B., 2001. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences* 22 (11), 573–580.
- McCarter, J.P., 2004. Genomic filtering: an approach to discovering novel antiparasitics. *Trends in Parasitology* 20, 462–468.
- Mejia, M.E., Fernandez Igartua, B.M., Schmidt, E.E., Cabaret, J., 2003. Multispecies and multiple anthelmintic resistance on cattle nematodes in a farm in Argentina: the beginning of high resistance? *Veterinary Research* 34, 461–467.
- Moy, T.I., Conery, A.L., Larkins-Ford, J., Wu, G., Mazitschek, R., Casadei, G., Lewis, K., Carpenter, A.E., Ausubel, F.M., 2009. High-throughput screening for novel antimicrobials using whole animal infection model. *ACS Chemical Biology* 4 (7), 527–533.
- Narahashi, T., Zhao, X., Ikeda, T., Salgado, V.L., Yeh, J.Z., 2010. Glutamate-activated chloride channels: unique fipronil targets present in insects but not in mammals. *Pesticide Biochemistry and Physiology*. doi:10.1016/j.pestbp.2009.07.008.
- Pong, S.-S., Wang, C.C., 1980. The specificity of high affinity binding of avermectin B<sub>1a</sub> to mammalian brain. *Neuropharmacology* 19, 311–317.
- Sangster, N.C., 1999. Anthelmintic resistance: past, present and future. *International Journal of Parasitology* 29, 115–124.
- Schürmann, S., Harder, A., Schnieder, T., von Samson-Himmelstjerna, G., 2007. Effects of Emodepside on egg hatching, larval development and larval motility in parasitic nematodes. *Parasitology Research* 101 (S1), 45–56.
- Scott, J.G., 1989. Cross resistance to the biological insecticide abamectin in pyrethroid-resistant house flies. *Pesticide Biochemistry and Physiology* 34 (1), 27–31.
- Silver, K.S., Song, W., Nomura, Y., Salgado, V.L., Dong, K., 2010. Mechanism of action of sodium channel blocker insecticides (SCBIs) on insect sodium channels. *Pesticide Biochemistry and Physiology*. doi:10.1016/j.pestbp.2009.09.001.
- Tomizawa, M., Casida, J.E., 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annual Review of Pharmacology and Toxicology* 45, 247–268.
- Toutain, P.L., 2002. Pharmacokinetic/pharmacodynamic integration in drug development and dosage-regimen optimisation for veterinary medicine. *AAPS PharmSci* 4 (4) (Article 38).
- Vais, H., Williamson, M.S., Devonshire, A.L., Usherwood, P.N.R., 2001. The molecular interactions of pyrethroid insecticides with insect and mammalian sodium channels. *Pest Management Science* 57, 877–888.
- Walters, W.P., Stahl, M.T., Murcko, M.A., 1998. Virtual screenings – an overview. *Drug Discovery Today* 3, 160–178.
- Wolstenholme, A.J., Rogers, A.T., 2005. Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology* 131, S85–S95.
- Woods, D.J., Williams, T.M., 2007. The challenges of developing novel antiparasitic drugs. *Invertebrate Neuroscience* 7 (4), 245–250.
- Wrigley, J., McArthur, M., McKenna, P.B., Mariadass, B., 2006. Resistance to a triple combination of broad-spectrum anthelmintics in naturally-acquired *Ostertagia circumcincta* infections in sheep. *New Zealand Veterinary Journal* 54 (1), 47–49.